PROCHELATORS FOR THE PREPARATION OF RADIOMETAL LABELED MOLECULES HAVING IMPROVED BIOLOGICAL PROPERTIES

The present invention relates to a convenient synthesis of novel bifunctional prochelators for coupling to bioactive peptides for radiometal labeling and to the radiometal labeled peptides that can be prepared while using these novel prochelators.

DOTA (1,4,7,10-tetrakis(carboxymethyl)-1,4,7,10 tetraazacyclo dodecane) and its derivatives constitute an important class of chelators for biomedical applications as they accommodate very stably a variety of di- and trivalent metal ions. $Gd(DOTA)^{-1}$ is an important MRI (Magnetic Resonance Imaging) contrast agent and as bifunctional versions DOTA is used in radioimmunotherapy.

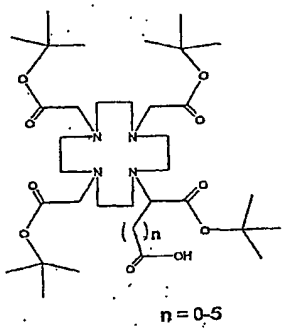
An emerging area is the use of chelator conjugated bioactive peptides for labeling with radiometals in different fields of diagnostic and therapeutic nuclear oncology. For their convenient and high yield synthesis, prochelators (compounds which become chelators upon deprotection) are necessary which are compatible with the solid and solution phase peptide synthetic procedures. Up till now such prochelators have not been available.

It is therefore the object of the present invention to provide a novel class of prochelators which are compatible with solid and solution phase peptide synthesic procedures, which prochelators can be used for the coupling of chelators to bioactive effector molecules, such as peptides.

According to the invention bifunctional macrocyclic synthons (prochelators) are provided based on DOTA, TRITA, TETA or structures comprising not 4, but 5 or 6 N atoms, which synthons are differentially protected and compatible with solid phase peptide synthesis procedures for labeling with hard Lewis acid radiometals.

Accordingly, the invention relates to polyazamacrocyclic compounds for radiometal labeling, comprising an N_n system, wherein n is 4, 5 or 6, with varying ring size, and wherein at least one of the N atoms is substituted with a free carboxylate group for coupling to an amino function in a bioactive effector molecule, while all N atoms carry a protected sidechain. After coupling of the bioactive effector molecule the protected sidechains can be deprotected to expose the chelating functions for labeling.

Particular compounds of the invention have the general formula:



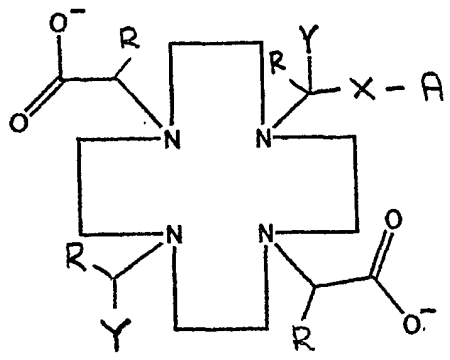
An example of such a compound is 1-(1-carboxy-3-carbotertbutoxypropyl)-4,7,10(carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAGA(tBu)4).

Compounds of the invention are prepared as follows. Starting from a relevant amino acid, the α-bromo-derivative thereof is synthesized. This derivative is orthogonally protected (tBu, Bzl). This alkylating agent will be reacted with cyclen, cyclam etc. to form a 1:1 adduct followed by tris-alkylation with bromoacetic acid tert-butylester and catalytic hydrogenation with H_2/Pd.

The synthon is monoreactive, carrying a free carboxylate group for coupling to the N-terminal end of the peptide and can be coupled to any biomolecule which then after deprotection can be labeled with a multitude of radiometals known to the person skilled in the art.

The invention relates more in particular to chelating compounds for radioactive labeling of bioactive

molecules, which chelating compounds have the general formula:



in which:

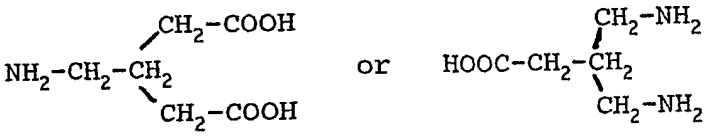
both Y groups may be positioned either trans as shown or cis;

A is an effector molecule, such as a peptide, in particular octreotide, CCK, substance P, gastrine, a protein, in particular an antibody or enzyme, sugars or radiosensitizing agents, like doxorubicin;

R is a hydrogen, a C_1-C_3 alkyl or an alcohol;

X is a spacer, in particular $(CH_2)_n$-X', in which n is 1-10 and X' is COOH, NH_2, SH, OH or O-halogen, in which halogen is in particular Br, I or Cl

or X is a molecule of the formula



Y is COO^-, CH_2CONH_2, CH_2CH_2OH.

In principle, the invention relates to every possible combination of the above identified substituents whether or not they are explicitly described herein in a specific combination or not.

Instead of an N_4 system the compound may comprise a N_5 or N_6 system and can be used for different natural and unnatural amino acids, such as protected cystein etc. The list of natural and unnatural amino acids is known to the person skilled in the art.

One of the new DOTA-based bifunctional prochelators, is 1-(1-carboxy-3-carbotertbutoxypropyl)-4,7,10(carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAGA(tBu)4 (**6d**)), which serves herein as a model compound. It should be noted that other compounds of the invention can be prepared in an analogous manner. The invention is not limited to compound **6d** alone.

Other embodiments of the invention are listed in Table 3.

The 5 step synthesis of **6d** is described in the Examples. It has an overall yield of about 20%. The coupling of **6d** to a bioactive peptide on solid phase is exemplified with use of a CCK-B (cholecystokinin) analogue.

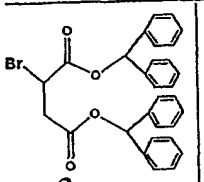
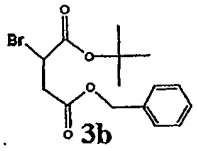
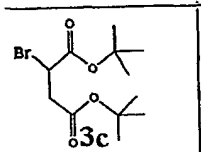
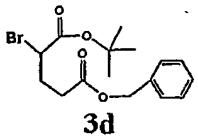
Described herein are the synthetic steps towards bifunctional orthogonally protected prochelators for coupling to the N-terminus of bioactive peptides or other useful amino functions in biomedical applications. The DOTA-derived chelator should provide 4 intact carboxylic acid functions besides the macrocyclic tetraazacyclododecane ring for a stable and efficient binding of metal ions and a function for biomolecule coupling.

The strategy includes the synthesis of an orthogonally protected bromo-alkyl-dicarboxylic acid diester for the monoalkylation of cyclen(1,4,7,10-tetraazacyclododecane). The synthesis of compound **6** (n=1, 2) is a 5 step procedure starting from the commercially available aspartic (**1b**) or glutamic acid-4-(5) benzyl ester (**1d**) (Scheme 1) using a method analogous to Holmberg (Chemische Berichte 1927, 60, 2197-2205) followed by tert-butylation using tert-butyltrichloro-acetimidate (TBTA) as reagent (Armstrong, A. et al., Tetrahedron Lett. 1988, 29, 2483-2486).

The monoalkylation of cyclen, the crucial step, showed strongly differing yields depending on the bromo-alkyl-dicarboxylic acid diester (**3a-d**) used (Table 1).

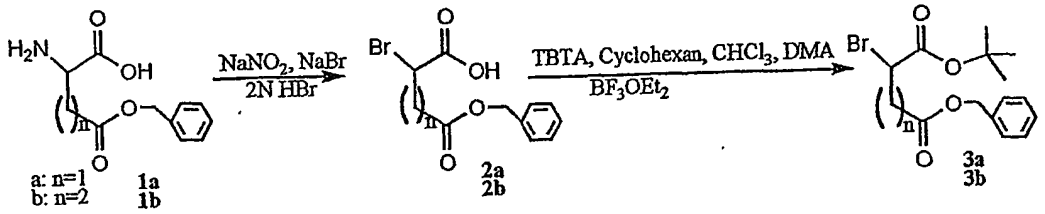
Table 1

Mono-alkylation yields of cyclen with different bromodicarboxylic acid esters

alkyl. agent	yield
 3a	79%
 3b	21%
 3c	<5%
 3d	83%

Scheme 1:

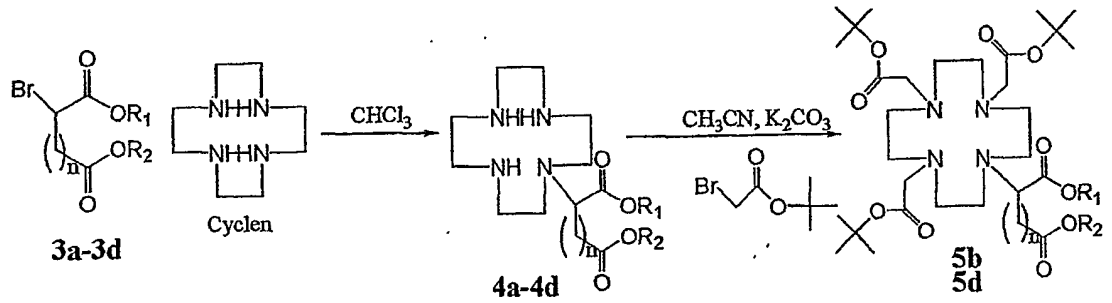
Synthesis of α-bromosuccinic acid-1-tertbutylester-4-benzyl ester (**3b**) and α-bromoglutaric acid-1-tertbutyl ester-5-benzyl ester (**3d**).



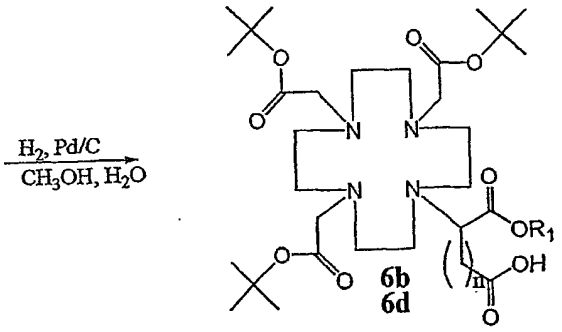
In earlier studies (André et al., Chem. Eur. J. 1999, 5, 2977-2982) the strategy was to use metals as protecting groups. In that work it was attempted to introduce succinic acid-di-tert-butylester (**3c**) and yields below 5% were found for the monoalkylation with the elimination product fumaric acid-di-tert-butylester as the main product. Interestingly the corresponding diphenylmethyl diester (**3a**) gave high monoalkylation

yields and negligible elimination. With the homologous 2-bromoglutaric-1-tertbutyl-5-benzylester (3d), no elimination product was found, obviously because no conjugated pi-system could be formed.

The remaining nitrogens were alkylated by use of three equivalents of bromoacetic acid-tertbutyl ester in $CHCl_3/K_2CO_3$.



Deprotection of the benzyl ester group was performed with H2/Pd/C.



a: R_1; R_2=Bzh; n=1
b: R_1=tBu; R_2=Bn; n=1
c: R_1;R_2=tBu; n=1
d: R_1=tBu; R_2=Bn; n=2
Bzh: diphenylmethyl-

The overall yield of 1-(1-carboxy-3-carbotertbutoxy-propyl)-4,7,10(carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTAGA(tBu)$_4$) (**6d**) over 5 steps was about 20% and of 1-(1-carboxy-2-carbotertbutoxyethyl)-4,7,10(carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTASA(tBu)$_4$) (**6b**) only about 2%.

The convenient use of **6d** is exemplified by its coupling to the CCK-B analogue D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH2 (**7**) attached to Rink-amide resin using HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyl-uroniumhexafluorophosphate) as coupling reagent. After deprotection, (18h, rt, TFA:phenol:thioanisol:water 85:5:5:5) DOTAGA-7 was obtained in high yield and showed

superior properties in comparison to other radiolabeled CCK-B analogues.

According to the invention it was thus found that the new type of prochelators in general and prochelator **6d** in particular, have widespread utility in the field of metalloradiopeptides, other radiolabeled biomolecules and for the synthesis of Gd^{3+} based MRI contrast agents. DOTAGA will allow to label with different radiometals for both diagnostic (^{111}In, $^{67/68}Ga$) and internal radiotherapeutic applications (^{90}Y, ^{177}Lu).

The present invention will be further illustrated in the examples that follow. Synthetic routes for the preparation of the compounds of the invention are given in Figures 1-3.

EXAMPLES

EXAMPLE 1

Typical procedure for the reaction of **1** to **2**

To a solution of 6 g (25.9 mmol) L-glutamic acid-5benzylester (**1d**) and 9.1 g (88.5 mmol) sodium bromide in 45 ml aqueous 1N hydrobromic acid (46 mmol) cooled to 0°C was added portionwise 3.175 g (46 mmol) sodium nitrite. After stirring for 2h at 0°C 2.25 ml concentrated sulfuric acid was added followed by diethylether.

The water phase was extracted 3 times with diethylether. The combined organic phases were extracted 4 times with brine, dried over Na_2SO_4 and concentrated.

The crude product was purified by chromatography (silica gel 60; hexane/EtOAc 3:1 to 2:1) and obtained as a yellow oil in a yield of 4.8 g (63%). ^1H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 10.1 (1H, COO$\underline{H}$; 7.3 (m, 5H, Ar); 5.15 (s, 2 H, C$\underline{H}_2$-Ph); 4.4 (dd, ^3J= 5.7, 1H, C$\underline{H}$Br); 2.6 (t, ^3J = 6.8, 2H, C$\underline{H}_2$-COOBzl); 2.5-2.2 (m, 2H, CHBr-C$\underline{H}_2$-CH_2); ^{13}C-NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 174.5 ($\underline{C}$OOH); 171.9 ($\underline{C}$OOBzl); 135.5 ($CH_2\underline{C}$(Ar)); 128.6, 128.4, 128.3 $\underline{C}$(Ar)); 66.8 (O-$\underline{C}H_2$-Ar); 44.1 (H$\underline{C}$Br); 31.4 (HCBr-$\underline{C}H_2$);

29.4 ($\underline{C}H_2COOBzl$; EI-MS m/z (intensity): 302, 300 (12, $[M]^+$); 91 (100, $[Bzl]^+$).

EXAMPLE 2

Typical procedure for the reaction of 2 to 3

To a solution of 4.8 g (15.9 mmol) **2d** in 20 ml $CHCl_3$ a solution of 6.26 ml (34.1 mmol) TBTA (tert-butyltrichloroacetimidate) in 20 ml cyclohexane was added dropwise over 20 min. During the addition a white precipitate formed, which was dissolved by the addition of 3.5 ml of DMA followed by 320 µl boron trifluoride ethyl etherate as catalyst. The reaction mixture was stirred for 3 days at Room Temperature (RT).

The mixture was concentrated and the remaining DMA phase was extracted 3 times with 30 ml hexane. The hexane phase was evaporated and the residue chromatographed over silica gel 60 (Hexane/EtOAc 20:1 later 9:1) affording 3.5 g (61%) of a colourless liquid. ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 7.4 (m, 5H, Ar); 5.15 (s, 2H, $C\underline{H}_2$-Ph); 4.35 (dd, 1H, $C\underline{H}Br$); 2.6 (td, 2H, $C\underline{H}_2$-COOBzl); 2.5-2.2 (m, 2H, CHBr-$C\underline{H}_2$-CH_2); 1.5 (s, 9H, $C(C\underline{H}_3)_3$). ^{13}C-NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 172.4 $\underline{C}$OOBzl); 168.7 ($\underline{C}$OOtBu); 136.1 (CH2$\underline{C}$(Ar)); 129.0, 128.8, 128.7 ($\underline{C}$(Ar)); 83.1 ($\underline{C}(CH_3)_3$); 67.0 (O$\underline{C}H_2$-Ar); 47.1 (H$\underline{C}$Br); 32.0 (HCBr-$\underline{C}H_2$); 30.1 ($\underline{C}H_2$COOBzl); 28.1 ($C\underline{C}H_3)_3$); EI-MS m/z (intensity): 302, 300 (18, $[M-C_4H_9]^+$); 57 (100, $[C_4H_9]^+$).

EXAMPLE 3

General procedure of the monoalkylation of cyclen

A solution of 870 mg (2.44 mmol) a-bromoglutaric acid-1-tert-butylester-5-benzylester (**3d**) in $CHCl_3$ was added dropwise over a period of 1h to a solution of 885 mg (4.9 mmol) cyclen in 4 ml $CHCl_3$. The mixture was stirred for 2 days at room temperature and concentrated to a brown oil. The crude product was purified by column chromatography (silica gel 60;

ethanol/NH_3 95:5), yield 920 mg (83%) of a colourless oil. ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 7.35 (m, 5H, Ar); 5.1 (s, 2H, $C\underline{H}_2$-Ph); 3.25 (dd, 1H, $C\underline{H}Br$); 2.9-2.5 (m, 18H, NCH_2, $CH_2COOBzl$); 2.2-1.85 (m, 2H, CHN-$C\underline{H}_2$-CH_2); 1.45 (s, 9H, $C(C\underline{H}_3)_3$); ^{13}C-NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 173.1 ($\underline{C}OOBzl$); 171.5 ($\underline{C}OOtBu$); 135.8 ($CH_2\underline{C}(Ar)$); 128.5, 128.3, 128.2 ($\underline{C}(Ar)$); 81.4 ($\underline{C}(CH_3)_3$); 66.2 (O-$\underline{C}H_2$-Ar); 63.5 ($H\underline{C}NCH_2$); 48.8, 48.0, 46.5, 45.6 (NCH_2CH_2N); 30.6 ($\underline{C}H_2COOBzl$); 28.2 ($C(\underline{C}H_3)_3$); 24.5 (HCN-$\underline{C}H_2$); EI-MS m/z: (intensity): 449.3 (56, $[M+H]^+$; 245.8 (100, [M+ $CH_3CN+2H]^{++}$).

EXAMPLE 4

Synthesis of 1-(1-carbobenzyloxy-3-carbotertbutoxy-propyl)-4,7,10-(carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane (5d)

A suspension of 1.1 g (5.6 mmol) bromoacetic acid-tert-butylester, 1.02 g (2.27 mmol) 1-(1-carbo-benzyloxy-3-carbotertbutoxypropyl)1,4,7,10-tetraaza-cyclododecane (**4d**), and 2.63 g (19.1 mmol) of dry potassium carbonate in 10 ml dry acetonitrile was stirred for 18 h at RT and was filtrated afterwards over Celite and evaporated to dryness.

The crude product was purified by column chromatography (silica gel 60; CH_2Cl_2/EtOH 9:1 followed by EtOH/NH_3 95:5) yield 1.3 9 (73%) of a yellow oil (**5d**). ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 7.35 (m, 5H, Ar); 5.1 (s, 2H, $C\underline{H}_2$-Ph); 3.6-1.9 (m, 27H, CHN, NCH_2, $CH_2COOBzl$, $CHNC\underline{H}_2$-CH_2, $C\underline{H}_2COOC(CH_3)_3$); 1.45 (s, 36H, $C(CH_3)_3$); ^{13}C-NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 174.6 (COOBzl); 172.9, 172.8, 172.6 ($\underline{C}OOtBu$); 135.6 ($CH_2\underline{C}(Ar)$); 128.5, 128.3, 128.2 ($\underline{C}(Ar)$); 82.4, 81.8, 81.8 ($\underline{C}(CH_3)_3$); 66.3 (O-$\underline{C}H_2$-Ar); 55.8, 55.7, 55.4, 52.6, 52.3, 50.3, 48.5, 48.1, 47.1, 44.3 (13C, $H\underline{C}NCH_2$, $N\underline{C}H_2\underline{C}H2_N$, $\underline{C}H_2COOtBu$, $\underline{C}H_2COOBzl$); ($NCH\underline{C}H_2CH_2$); 28.0, 28.0, 27.8, 27.6 ($C(\underline{C}H_3)_3$); EI-MS m/z (intensity): 813.6 (22, $[M+Na]^+$); 791.6 (38, $[M+H]^+$); 396.5 (100, $[M+2H]^{++}$).

EXAMPLE 5

Synthesis of DOTAGA(tBu)4 (6d)

600 mg (0.76 mmol) **5d** was dissolved in methanol, and 30 mg Pd/C suspended in 1 ml H_2O was added. The mixture was hydrogenated for 2 days, filtrated over Celite and evaporated to dryness. The crude product was chromatographed on silica gel 60 ($EtOH/NH_3$ 95:5) to obtain 470 mg (84.6%) of a white solid (**6d**). ^{1}H-NMR (300 MHz, $CDCl_3$, $SiMe_4$): 6.5 (br, 1H, COOH); 3.6-2.0 (m, 27H, CHN, NCH_2, $C\underline{H}_2COOH$, CHN-$C\underline{H}_2$-CH_2, $C\underline{H}_2COOC(CH_3)_3$); 1.45 (s, 36H, $C(CH_3)_3$); ^{13}C-NMR (75 MHZ, $CDCl_3$, $SiMe_4$): 175.2 ($\underline{C}OOH$); 175.0, 172.9, 172.8, 172.6 ($\underline{C}OOtBu$); 82.4, 82.1, 81.9 ($\underline{C}(CH_3)_3$); 55.8, 60.1 (N$\underline{C}$HCOOtBu); 55.9, 55.8, 55.6, 52.7, 52.6, 52.5, 48.6, 48.5, 48.2, 47.1, 44.3 (12C, N$\underline{C}H_2CH_2N$, $\underline{C}H_2COOtBu$, $\underline{C}H_2COOH$); 33.4 (NCH$\underline{C}H_2CH_2$); 27.9, 27.8 ($C(\underline{C}H_3)_3$); EI-MS m/z (intensity): 723.5 (27, $[M+Na]^+$); 701.5 (68, $[M+H]^+$); 351.4 (100, $[M+2H]^{++}$).

EXAMPLE 6

Preparation of DOTAGA-7

Compound **6d** was coupled to the CCK-B analogue D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH2 (**7**) attached to Rink-amide resin using HATU (O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate) as coupling reagent.

After deprotection, (18h, rt, TFA:phenol:thioanisol:water 85:5:5:5) DOTAGA-7 was obtained in high yield and showed superior properties in comparison to other radiolabeled CCK-B analogues.

The data of the DOTAGA-CCK-B analogue (DOTAGA-7) were as follows: Yield: 12.7 mg, HPLC purity >95%, (+) EI-MS m/z (intensity): 1486.1 (48, $[M+H]^+$); 743.7 (60, $[M+2H]^{++}$); (-) EI-MS m/z (intensity): 1484.0 (28, $[M+H]^-$); 741.8 (90, $[M+2H]^{--}$).

EXAMPLE 7

Biological properties

It has been found that when using the prochelators of the invention for preparing biologically active molecules, these molecules have better biological properties than molecules prepared with other (pro)chelators. The advantages for example are a better labeling yield according to the following table 2:

Table 2

	amount of radioactive label	labeling efficiency of DOTATOC-peptide	labeling efficiency of DOTA3-peptide (DOTAGA) (invention)
5 µg of the compound to be labeled	5 mCi ^{90}Y	99.5%	99.9%
	10 mCi ^{90}Y	95%	100%
	20 mCi ^{90}Y	92%	99.9%

In addition, the stability is higher. The reaction of ^{90}Y-DOTATOC or ^{90}Y-DOTAGA at 37°C with the chelator DTPA results in ^{90}Y-DTPA. The half life of this reaction is 23 hours for DOTATOC and 79 hours for DOTAGA.

Table 3 shows various biological properties of the compound of the invention Y-DOTA3TOC and Y-DOTAta.13TOC. The radiometal is not shown.

Biological results of Yttrium labelled Peptides

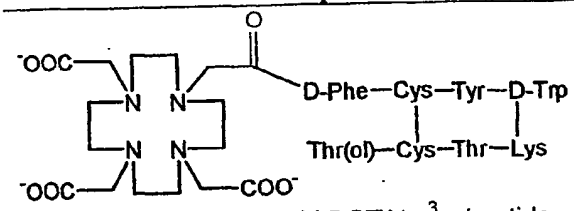
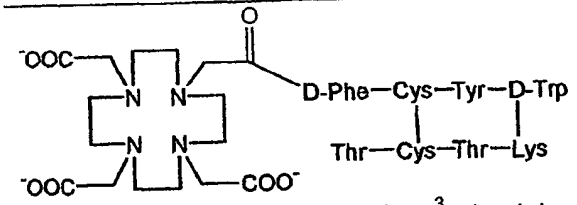
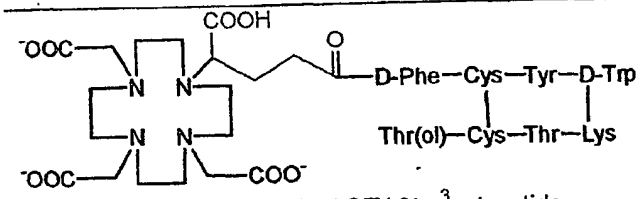
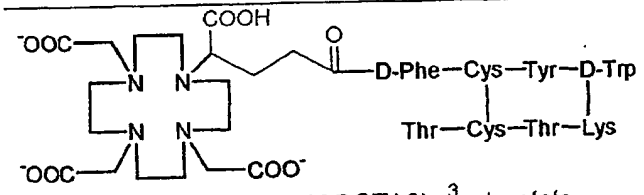
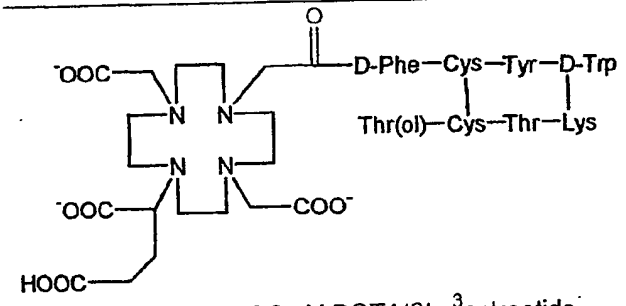
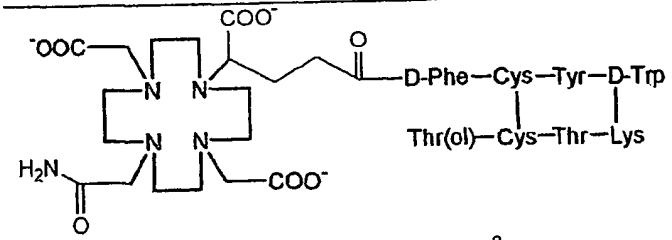
Peptide	IC_{50}(hsst2)	Tumor	Kidney	Charge
 Y-DOTATOC: Y-DOTAtyr^{3}octreotide	11 ± 1.7	13.5	12.3	+1
		adrenal, pancreas low		
 Y-DOTATATE: Y-DOTAtyr^{3}octreotate	1.6 ± 0.4	14.5	8	0
 Y-DOTA3TOC: Y-DOTA3tyr^{3}octreotide	1.5 ± 0.5	30	56	0
 Y-DOTA3TATE: Y-DOTA3tyr^{3}octreotate	3.5	13.5	68	-1
		adrenal, pancreas high		
 Y-DOTAt3TOC: Y-DOTAt3tyr^{3}octreotide	28 ± 10	23.5	25.5	0
 Y-DOTAta.13TOC: Y- DOTAta.13tyr^{3}octreotide	0.23			+1

Table 3